**SHORT** (250 words) **208 WORDS**

AUTHORS:

Rubio-Martinez LM1, Rioja E1, Castro Martins M1, Saengsoi W2, Clegg P2, Peffers MJ2. 1Dept. Equine Clinical Studies, Institute of Veterinary Science, University of Liverpool, United Kingdom. 2Dept. of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, United Kingdom.

TITLE:

**Local anaesthetics but not morphine or magnesium sulphate are toxic for equine chondrocytes and synoviocytes *in vitro*.**

ABSTRACT BODY:

*Introduction*

Chondrotoxic effects of local anaesthetics have been reported but knowledge on their toxic effects on synoviocytes or their effect on the cellular production of inflammatory cytokines is limited. The purpose of this study was to evaluate the *in vitro* effects of local anaesthetics, morphine, or magnesium sulphate (MgSO4) on the cell viability and pro-inflammatory cytokine gene expression of equine synoviocytes and chondrocytes.

*Material and Methods*

Equine synoviocytes and cartilage explants harvested from normal metacarpophalangeal joints were exposed, in a co-culture system, to mepivacaine, bupivacaine, morphine or MgSO4 alone or in combination. Chondrocyte and synoviocyte cell viability was assessed by CellTiter-Glo Luminescent Cell Viability Assay. Synoviocyte gene expression of IL-1β, IL-6 or TNF-α was measured and compared using the ∆∆ct method and normalised to the housekeeping gene GAPDH. Statistical significance was tested using general linear models with Dunnet’s comparisons was and considered when *p* <0.05.

*Results*

Mepivacaine or bupivacaine decreased cell viability and increased the gene expression of IL-1β, IL-6 or TNF-α. However, morphine or MgSO4 alone did not.

*Discussion/Conclusion*

Single short exposure to local anaesthetics is toxic to both chondrocytes and synoviocytes; however, morphine or MgSO4 did not demonstrate cytotoxic effects. Further investigation on the suitability and indications of morphine or MgSO4 as intra-articular drugs are warranted.

**LONG (1500 words) 1022 words**

**Introduction**

Intra-articular injections of local anaesthetics are commonly performed in horses and other species to determine sources of pain and as perioperative pain control. Despite their widespread use, there is growing concern over the potential toxicity of these substances and their long-term effects on articular tissue1. Chondrotoxic properties of local anaesthetic agents have been reported in humans and animals, but the effects on synoviocytes are still largely unknown. Morphine and magnesium sulphate (MgSO4) provide articular analgesic and anti-inflammatory effects when administered intra-articularly with apparently minimal toxic effects on human and canine chondrocytes1-3.

The purpose of this study was to evaluate the *in vitro* effects of clinically-relevant doses of local anaesthetics, morphine or MgSO4 on chondrocyte and synoviocyte viability and gene expression in an equine co-culture *in vitro* model. We hypothesised that local anaesthetics would produce deleterious effects on chondrocyte and synoviocyte viability and increase the expression of pro-inflammatory cytokines.

**Materials and methods**

Synoviocytes and cartilage explants obtained from metacarpophalangeal joints of 10 grossly normal, skeletally mature horses (6-10 years old) were exposed in a co-culture system to the different treatments. Treatments included: control (standard culture medium containing 10% foetal calf serum), mepivacaine 4.4 mg/ml, bupivacaine 2.2 mg/ml, morphine 2.85 mg/ml, magnesium sulphate 37 mg/ml. Concentrations were based on the average volume of synovial fluid in the equine metacarpophalangeal joint and the doses commonly used in clinical practice. Exposure time was based on the reported half-life for the local anaesthetics in horses. After exposure, synoviocyte and chondrocyte viability was assessed with CellTiter-Glo Luminescent Cell Viability Assay4; 5. Real-time Quantitative Polymerase Chain Reaction (qPCR) analysis was used to measure relative gene expression of Interleukin 1 beta (IL1β), Interleukin 6 (IL6) and Tumour Necrosis Factor alpha (TNFα) relative to glyceraldehydes-3-phosphatedhyrogenase (GAPDH).

*Statistical analysis*

Data were analysed using commercially available statistical software (SPSS, version 22.0, 2013, Chicago, USA). Graphical displays and Anderson-Darling test were used to check for departures from assumptions of normality. Log transformations were applied when data were non-normally distributed. Gene expression data were normalised to the housekeeping gene using the ∆∆ct method 6 and statistical analysis applied to the 2^-∆CT values. General linear models with Dunnet’s comparisons with control group was undertaken and significance considered when *p* <0.05.

**Results**

*Cell viability*

Compared with control group, viability was significantly reduced in groups exposed to mepivacaine for chondrocytes and synoviocytes, and for synoviocytes in groups mepivacaine and bupivacaine. Exposure to morphine or MgSO4 did not have a significant effect on cell viability for either cell type.

*Real-time qPCR*

Treatment with morphine or MgSO4 did not have an effect on synoviocyte gene expression of IL-1β, IL-6 or TNF-α. Gene expression of IL-1β increased in treatment group mepivacaine. Gene expression of IL-6 and TNF-α increased in groups mepivacaine and bupivacaine.

**Discussion**

Chondrotoxic effects of local anaesthetics are reported in the literature and results of this study confirm that a single short exposure to local anaesthetics can cause significant articular cytotoxic effects characterised by decreased viability and increase the expression of pro-inflammatory cytokines by not only chondrocytes but also synoviocytes.

In this study mepivacaine appeared more toxic than bupivacaine; however, in previous studies mepivacaine was less toxic than bupivacaine on human7 and equine8 chondrocytes on monolayer cultures. Local anaesthetics have drug-, dose- and time-dependent cytotoxic effects7 and the bupivacaine concentration used in the present study was lower than in previous studies. Mepivacaine has been previously associated with chondrotoxic effects on human cartilage7 and equine chondrocytes9.

The present study corroborates the absence of deleterious effects of morphine or MgSO4 on equine chondrocyte viability *in vitro*2; 10, and extends this absence of toxicity to articular synoviocytes. In addition, no effects on expression of pro-inflammatory cytokines were observed when tissues were exposed to morphine and/or MgSO4. Anti-inflammatory effects of morphine or morphine on articular tissues have been shown in different species including horses10-12.

In conclusion, local anaesthetics have toxic effects not only on chondrocytes but also synoviocytes. This study provides further evidence to support the use of morphine and MgSO4 as intra-articular drugs. Further investigation on the suitability and indications of morphine or MgSO4 as intra-articular drugs are warranted.

**References**

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